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Review Article

TP53 Status and Response to Treatment in Breast Cancers

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The p53 wild-type protein plays an important role in cells as is shown by its fine regulation at different levels. Since its discovery, numerous mutations have been described. In breast cancers, p53 is mutated in almost 30% of cases, with a higher frequency in some tumor subtypes. *TP53* mutation is reported to be a factor for good prognosis in some studies, while in others it is a factor for poor prognosis. The explanation for these different results could be linked to the fact that the studies were performed on different tumor types and with different therapy regimens.

1. Introduction

Breast cancer affects more than one million patients annually in the world and is a leading cause of mortality [1]. The prognosis of patients with localized breast cancer is determined by clinical and biological factors such as age at diagnosis, tumor size, nodal status, tumor histological grading, expression of estrogen/progesterone receptors [2], and Her2 status. Treatments combine surgery on the primary tumor, radiation therapy of the breast, chemotherapy, and hormone therapy.

In adjuvant setting, the benefit of chemotherapy has been confirmed since the 1980s by the 5-yearly meta-analyses of the Early Breast Cancer Trialists' Collaborative Group (EBCTCG), particularly in women under 50 years [3]. Adjuvant regimens include mainly anthracyclines and taxanes [4]. In patients with localized breast cancer overexpressing Her2, trastuzumab has been proven to significantly increase survival [5]. In patients with estrogen receptor (ER-) positive tumors, addition of hormone therapy such as tamoxifen or aromatase inhibitors significantly decreases the risk of recurrence and increases survival [3, 6].

The same chemotherapeutic agents and hormone therapies are currently used for patients with metastatic disease.

Despite a clear benefit of these medical treatments in terms of survival, median overall survival ranges from 31–39 months [7, 8]. In addition, 20–40% patients exhibit primary resistance to medical treatment [7–9] suggesting that we need better knowledge of the predictive factors of response to treatment (chemotherapy, hormone therapy, radiotherapy). In this paper, we review the relationship between *TP53* status in breast cancers and response to treatment.

2. The Biology of *TP53*

In 1979, three teams led by A. Levine, P. May and L. Old discovered the p53 protein, a protein that is, highly conserved across animal species, which is encoded by the *TP53* gene located on the short arm of chromosome 17 (17p13.1). Its sequence, about 20 Kb, contains 11 exons, but the first exon does not encode and is located about 10 Kb from other exons [10]. In 1989, Vogelstein's team discovered that the *TP53* gene is inactivated in human cancers [11].

The p53 protein contains 393 amino acids (AA), is divided into regions highly conserved during evolution [12], and its role in numerous regulatory mechanisms has been well established. The protein is composed of: (i) an N-terminal region (AA 1–42), (ii) a region rich in proline

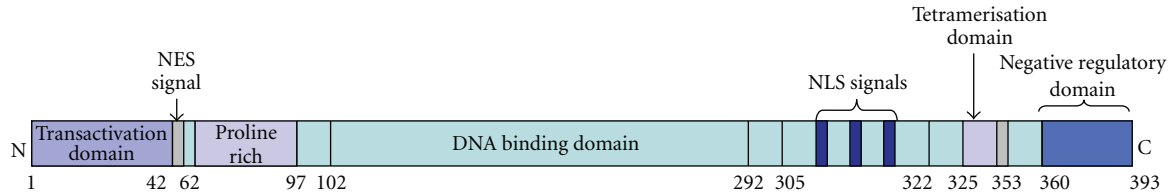


FIGURE 1: The p53 protein structure. The N-terminal region contains the transactivation domain (AA 1–62) and a proline-rich region (residues 63–97) with a role in apoptosis. The central domain (the core domain, AA 102–292) contains specific DNA sites. The C-terminal region includes the tetramerization domain (AA 325–360) and a negative autoregulatory domain. NES signals exist on both N- and C-terminal, whereas NLS signals are located on C-terminal region.

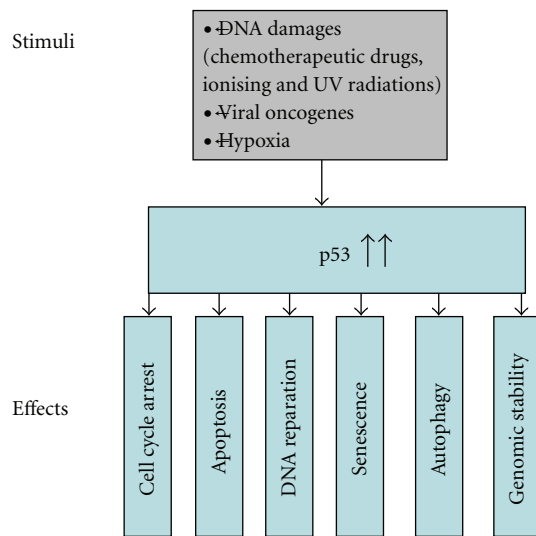


FIGURE 2: Stimuli and effects of activation of p53 protein. In response to diverse stimuli p53 functional protein induces diverse effects such as cell cycle arrest, apoptosis, repair of DNA lesions, senescence, and autophagy.

residues (AA 63–97) involved in the induction of apoptosis [13], (iii) a core domain necessary for binding to DNA (AA 102–292), containing most of the inactivating mutations found in different types of human cancers [14], (iv) a tetramerization domain (AA 323–356), and (v) a C-terminal region (AA 363–393). This C-terminal region of p53 binds to the N-terminal domain of Mdm2 (murine double minute 2). In addition, there are also sequences for exporting to the cytoplasm at the N- and C-terminal ends (NES, nuclear export signal), as well as nuclear localization sequences at the C-terminal end (NLS, nuclear localization signal), enabling the regulation of subcellular localization of p53 [15, 16] (Figure 1).

3. Stimuli and Activation Effects of TP53

Multiple stimuli such as ionizing radiations, DNA lesions, nitric oxide, hypoxia, chemotherapeutic agents, or oncogenic stimuli can activate p53 [17, 18] (Figure 2). In response to various stimuli, p53 undergoes different changes and this

activation could induce different effects. p53 is a transcription factor involved in the control of G₁/S and G₂/M phase transition, in DNA repair, and in induction of senescence, apoptosis, autophagy, mitotic catastrophe, and angiogenesis.

3.1. Cell Cycle Regulation. TP53 regulates the control of the G₁ checkpoint and can induce an arrest of the cell cycle, repair or apoptosis if DNA lesions are extensive [19]. Wild-type p53 protein can transcriptionally transactivate *p21^{Cip1}*, a potent inhibitor of most cyclin-dependent kinases, involved in the cell cycle arrest [20]. p53 also stimulates the expression of the 14-3-3 σ protein that sequesters the cyclin B1/CDK1 complex to block the transition G₂/M. But p53 also induces the expression of many other genes such as GADD45, which interacts with PCNA to inhibit the passage to S phase, or Reprimo to block the cell cycle in G₂ phase [21].

3.2. Cell Senescence. Cellular senescence is thought to play an important role in tumor suppression and to contribute to cellular aging [22]. The p53 tumor suppressor is also a critical mediator of senescence, and it seems to play a critical role in the induction and maintenance of cellular senescence. The first information about the importance of p53 on cell senescence was provided by the studies using T antigens of SV40 virus which inactivate p53. p53-null fibroblasts remain immortal when propagated *in vitro*. p53 activation is an essential step in the induction of senescence following DNA damage or other forms of stress. In the context of senescence, p53 is controlled by ATM/ATR and Chk1/Chk2 proteins which cause the posttranslational stabilization of p53 through its phosphorylation [23].

3.3. Apoptosis. Apoptosis is one of the principal functions of p53. It has been shown that p53 can transactivate the cell death receptors CD95 or TNF which induce the formation of the DISC complex and finally activate caspase 8. p53 also activates proapoptotic members of the Bcl2 family: Bax, Noxa, and Puma-involved in the permeabilization of the outer mitochondrial membrane [24]. Moreover, p53 has also been reported to have a direct role in cell death initiation by localizing to mitochondria and regulating mitochondrial outer membrane permeabilization directly. Thus, p53

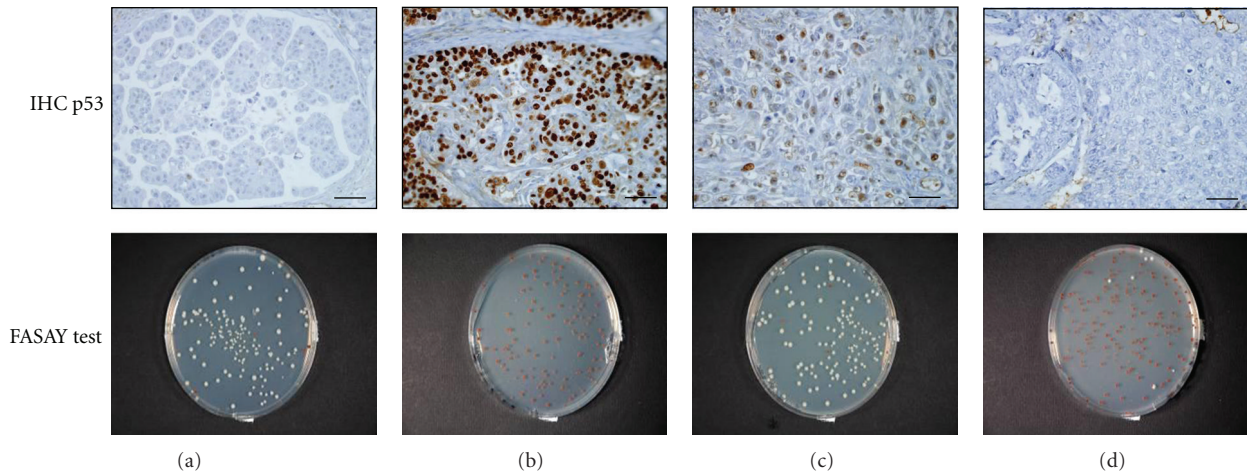


FIGURE 3: Detection of p53 by immunohistochemistry and determination of *TP53* status by FASAY test. Immunohistochemistry (IHC) is not the best method to determine p53 status. Thus, four different situations are illustrated: (a) p53 is negative on IHC and not mutated on FASAY test; (b) p53 is positive on IHC and mutated on FASAY test (red colonies); (c) p53 is weakly positive on IHC but not mutated on FASAY test (white colonies); (d) p53 is negative on IHC but mutated on FASAY test (for example codon Stop on exon 5); Magnification x400. p53 antibody (clone DO-7, DakoCytomation, France) was used.

protein can directly induce permeabilisation of the outer mitochondrial membrane by forming complexes with the protective BclXL and Bcl2 proteins, resulting in cytochrome C release [25, 26].

3.4. Autophagy. Autophagy is a process suppressing tumor initiation and reducing genomic instability. Autophagy consists in the lysosomal degradation of intracellular components leading to the generation of new metabolic substrates, thus favouring adaptation to stress and cell survival [27]. p53 can activate but also inhibit autophagy. Under stress, p53 can activate its target gene in the nucleus, such as AMPK β 1 and β 2 (AMP-activated protein kinase) [28], DAPK-1 (death-associated protein kinase1), and DRAM (damage-regulated autophagy modulator) [29]. Cytoplasmic, but not nuclear, p53 is able to repress autophagy [30, 31].

3.5. Mitotic Catastrophe. Mitotic catastrophe is a biological state that precedes cell death. In response to DNA damage, checkpoints are activated to delay cell cycle progression and to coordinate repair. Reports have suggested that the absence of p53 might increase mitotic catastrophe [32]. p53-deficient cells in an unchecked tetraploid G_1 state reduplicate their DNA, leading to polyploidy and subsequent chromosomal instability. In the presence of wild-type p53, the polyploidy causes the activation of p21^{CIP1} and an irreversible arrest in the cell cycle, or in cell death, thus preventing the propagation of aneuploidy [33].

3.6. Angiogenesis. The formation of new blood capillaries (angiogenesis) is closely regulated by proangiogenic and antiangiogenic factors [34]. The p53 protein has been shown

to limit angiogenesis by few mechanisms: (1) interfering with central regulators of hypoxia that mediate angiogenesis, (2) inhibiting the production of proangiogenic factors, and (3) directly increasing the production of endogenous angiogenesis inhibitors. The combination of these effects allows p53 to efficiently shut down the angiogenic potential of cancer cells [35]. Wild-type p53 plays a role in limiting tumor vascularization as demonstrated by some clinical studies [36]. Mutant p53 plays a central role in promoting angiogenesis in colon cancer progression [37], and tumors carrying p53 mutations are more highly vascularised than tumors harboring wild-type p53. The loss of *TP53* appears to amplify the HIF (Hypoxia Inducible Factor) pathway. HIF-1 α has been shown to be physically associated with p53 in immuno-precipitation experiments. p53 promotes MDM2-mediated ubiquitination and degradation of HIF-1 α , while loss of p53 leads to amplification of the HIF response [38].

4. Regulation of p53

The protein p53 can be regulated at different levels:

- (i) by posttranslational modifications, such as phosphorylation, sumoylation, or acetylation of the protein [39, 40],
- (ii) by increasing the protein concentration: one of the key regulators of p53 is Mdm2 which targets p53 for breakdown by the proteasome [41],
- (iii) by cellular localization: import and nuclear export is closely regulated because the functions of p53 depend on its nuclear localization. Efficient transfer to the cytoplasm depends on Mdm2 forming a complex with p53, which is why ubiquitin ligase activity of Mdm2 is essential for nuclear export of p53 [42].

The ubiquitination of p53 by Mdm2 occurs in the C-terminus domain, and it has been shown that mutations in lysine residues inhibit the nuclear export of p53 by Mdm2 [15].

5. Detection of p53

Under normal conditions, p53 protein remains undetectable due to its short half-life (Figure 3(a)). In contrast, mutant proteins accumulate in the nucleus of tumor cells due to increased half-life and an altered conformational structure (Figure 3(b)).

In a large majority of studies, detection of p53 is highlighted by the protein in the nucleus using immunohistochemistry techniques. This method of detection could give false positive results from stabilization of wild-type p53 proteins due to cellular stress (Figure 3(c)) or could give false negatives due to codon stop, frameshifts, or other destabilizing mutations (Figure 3(d)). Lack of immunostaining for p53 despite mutation of the *TP53* gene was particularly seen in tumors harboring nonsense mutations or deletions/splices [43] while other studies have shown that the identification of positivity for p53 solely detected by immunohistochemistry did not always reflect a p53 mutation [44].

Another way to determine *TP53* status is the FASAY test (Functional Analysis of Separated Alleles in Yeast) [45]. After the extraction of mRNA from whole blood or from tissue (normal or tumoral) reverse transcription by RT-PCR is carried out. The DNA binding domain is amplified and the PCR product is cloned by homologous recombination into yeast with a linearized expression plasmid vector carrying the 5' and 3' ends of the *TP53* open reading frame. The plasmid, thus, has a constitutive expression of human *TP53*. The yeast contains an open reading frame (ORF) for adenine regulated by a promoter under the control of *TP53*. The yeasts are selected on a selective medium lacking leucine, but containing adenine. When *TP53* is wild-type, a complete metabolism of adenine occurs and the colonies are white. The cells containing mutant *TP53* fail to express adenine, and, consequently, the colonies are red because of the accumulation of an intermediate adenine metabolite. These colonies are also smaller than normal because adenine limits growth. Thus, the *TP53* status can be easily determined by the color of transfected yeast cells [45].

Some studies analyzed *TP53* status in breast tumors using a robust and sensitive approach combining three different methods: p53 immunohistochemistry, FASAY test, and sequencing of the coding sequence. Tumors were considered *TP53* mutant when (i) more than 15% of the yeast colonies were red (ii) analysis using the split versions of the test could identify the defect in the 5' or 3' parts of the gene, and (iii) sequence analysis from mutant yeast colonies could identify an unambiguous genetic defect (mutation, deletion, splicing defects) [46]. FASAY provided a major contribution to the analysis by revealing several *TP53* mutations not detected by direct sequencing, principally in samples highly contaminated with stromal cells [47, 48].

6. *TP53* Mutations

The function of p53 is altered in nearly 50% of cancers, p53 being inactivated by mutations in the DNA binding domain or deletion of the carboxy-terminal domain [49]. In other cases, loss of p53 function is related to other mechanisms such as interaction with a viral protein (in lymphoma, hepatocellular carcinoma), multiplication of the *MDM2* gene (in sarcomas), and deletion of p14^{ARF} gene (in breast or lung tumors) [50].

p53 transcriptional activity is based on the formation of tetramers (dimers of dimers). Mutant proteins may interfere with wild-type p53 by forming hetero-oligomers that are less competent for specific DNA binding [51]. It has been shown that some missense mutations gain oncogenic properties. In the experimental procedure, the authors introduced common *TP53* cancer mutations R248W and R273H, into the humanized p53 knock-in allele in mice. They demonstrate that the tumour-suppressor functions of p53 were abolished in p53-mutant mice. Moreover, inter-chromosomal translocations were observed [52]. Mutants p53, mutants R248W, and R273H interact with Mre11 and inactivate Mre11/ATM-dependent DNA damage responses, leading to chromosomal translocation and defective G₂/M checkpoint [53]. The analysis of the spectrum of *TP53* somatic mutations in human cancers shows an association between exposure to different types of carcinogens and the development of various cancers. *TP53* mutations are mostly missense point mutations and are located in 80% to 90% of cases in the central region encoding the DNA binding domain [51, 54]. Some mutations are found much more often than others. Thus, the following six mutations account for about 30% of all mutations: 175Arg-His, 248Arg-Gln, 273Arg-His, 248Arg-Trp, 273Cys-Arg and 282Arg-Trp [13].

The universal mutation database (<http://www-p53.iarc>) contains 30,500 mutations [55]. Thus, mutations are found in 20–30% of malignant melanomas [56], in 50% of superficial bladder cancers, in 29% of nonsmall cell lung cancers [57], in 58% of hepatocellular carcinomas [58], and in 25% of breast cancers [59]. Thirty-seven percent of the myeloma patients with del (17p) present a *TP53* mutation versus 0% of patients lacking the del(17p), but the prognostic significance of these mutations remains to be evaluated [60]. The loss of p53 tumor suppressor activity is associated with a poor prognosis in mantle cell lymphoma [61].

7. *TP53* Status and Prognosis in Breast Cancer

Breast cancer is a heterogeneous disease. Histological type, grade, tumor size, lymph node involvement, and estrogen receptor and HER-2 receptor status, all influence prognosis and the probability of response to systemic therapies [62].

TP53 is mutated in about 30% of breast cancers [59]. The possible links between alterations of p53 and clinical or pathological features of breast tumors have been widely investigated.

The first study to examine gene-expression patterns of breast cancer suggested that at least four major molecular

classes of breast cancer exist: luminal-like, basal-like, normal-like, and HER-2 positive [63]. Basal-like breast cancers account for 15% of breast cancers and are often described as triple negative breast cancers (TNBCs). In fact, TNBCs, defined by lack of expression of estrogen receptor, progesterone receptor, and HER2 [64], probably include both basal-like breast cancers and some poorly differentiated luminal breast cancers [65]. They are also associated with a younger age and a poor prognosis [66]. TNBCs also have an increased frequency of *TP53* mutations [67]. Recently, it was shown that p53 status was a strongly unfavorable prognostic factor for relapse-free survival and overall survival only in a triple negative group in patients treated with adjuvant anthracycline-containing chemotherapy. Under this treatment, expression of p53 provides information concerning poor outcome in triple-negative tumors [67, 68].

HER2-like tumors show an increased expression of genes associated with *ErbB2* amplicon and *TP53* mutation [69]. The simultaneous immunodetection of p53/*ErbB2* appears to have greater negative prognostic relevance [70]. Other data are consistent with the hypothesis that certain *TP53* mutations and *ErbB2* overexpression are predictive of resistance to doxorubicin in patients with breast cancer [43].

Inflammatory breast cancer (IBC) is a clinical diagnosis known as the T4d category in the TNM classification [71]. It is a distinct clinical subtype of locally advanced breast cancer (LABC), with a particularly aggressive behaviour and poor prognosis [72]. *TP53* mutations are more frequent in inflammatory breast cancer (50%) than in noninflammatory breast cancer (20–30%) [73, 74]. Interpretation of prognostic data is complicated by the fact that earlier studies only used immunohistochemistry to detect the accumulation of p53. Breast tumors with positive immunostaining for p53 are usually ER and PR negative. This is often associated with a high rate of proliferation, a high histological grade, aneuploidy, and a poor prognosis [75, 76]. A prognostic value of *TP53* mutation by sequencing was found in more than 25 studies analyzing 6000 patients [46]. Moreover, the prognostic significance of *TP53* mutation depends on the specificity of the mutation [77].

7.1. p53 Status and Anthracycline Chemotherapy in Breast Cancer. In 50 noninflammatory locally advanced breast cancers that were treated with dose-intensive epirubicin-cyclophosphamide combination, eight complete pathological response (pCR) were shown in the 14 patients with tumors containing mutated *TP53*, whereas none of the 36 patients with a wild-type *TP53* status had a pCR after chemotherapy [78]. In 80 patients with noninflammatory breast cancers treated with front-line chemotherapy comprising epirubicin and cyclophosphamide, 28 had *TP53*-mutant tumors. Fifteen out of these 28 patients exhibited pCR while none of the 52 patients with *TP53* wild-type tumors had a pCR. Moreover, nine out of ten of the highly aggressive basal subtypes showed pCRs. This demonstrates that, in noninflammatory breast cancers, *TP53* status could be a key predictive factor for response to this chemotherapy treatment and further suggests that the

basal subtype is exquisitely sensitive to this association [79]. Research on stage II-III breast cancer patients treated front line with epirubicin-based regimens of various cyclophosphamide dose intensities suggest that cyclophosphamide dose intensification in ER negative and *TP53* mutated patients could significantly improve their response [80]. All these studies show an increased response in tumors with a mutation in *TP53*. Recently, it was shown on *in vivo* models that epirubicin-cyclophosphamide treatment induces senescence-like features in *TP53* wild-type tumor, probably accounting for cell cycle arrest and subsequent resistance to treatment. Conversely in *TP53* mutated tumors, chemotherapy induces mitotic catastrophe and tumor death, accounting for complete response to this association exclusively in patients with *TP53* mutated tumors [81].

In contrast, in a study on 63 patients with locally advanced breast cancer and treated with doxorubicin, a correlation was observed between the presence of mutations in the zinc finger domain of the p53 protein and resistance to treatment [82]. These results were confirmed in another study involving 90 patients [43]. Some clinical studies showed that mutant p53 confers chemoresistance in patients with breast cancer. Patients with missense mutations located in zinc-binding regions had significantly decreased disease-free and overall survival relative to patients whose tumors had mutations in other domains [83]. It has been suggested that codon polymorphism 72 (Arg/Pro) could affect the response to chemotherapy in tumor cells through the interaction between p53 and p73 [84]. Protein p73 belongs to the same family as p53 and p63 and shows a striking homology within both the DNA binding domain and oligomerization domain. P73 presents a wide array of splicing variants α , β , γ , δ , ϵ , ζ [85]. p73 has proapoptotic and antiapoptotic properties. p73alpha mRNAs encode two types of isoform (TAp73alpha and DeltaNp73alpha) resulting from the use of two different promoters, and eliciting or lacking NH(2)-terminal transactivation domain, respectively. DeltaNp73alpha inhibits p53 proapoptotic function [86]. Patients with breast cancer with a variant Pro/Pro *TP53* are less sensitive to anthracycline-based therapy than those with a variant Pro/Arg or Arg/Arg [87]. These studies show that mutations in *TP53* could induce a resistance to treatment based on anthracyclines.

These results are not contradictory, they rather result from studies exploring different tumor types and different regimens. *TP53* status may have a different predictive value for efficacy of anthracycline/alkylating agents-based regimen in each molecular subclass [88]. In 630 patients with breast cancer, the clinical outcome was significantly different for different *TP53* mutation types but also for different tumors [89].

7.2. p53 Status and Nonanthracycline Chemotherapy in Breast Cancer. In 67 tumors treated with 5-FU, epirubicin, cyclophosphamide, or paclitaxel, combined sequencing and immunohistochemistry showed a significant association between the presence of *TP53* mutation and response to paclitaxel. The efficacy of paclitaxel during mitosis is induced

by the fact that there is no stop in G₁ phase, because of absence of p53 [46]. Trastuzumab, an HER2-targeted monoclonal antibody, induces growth arrest and apoptosis in a p53-independent manner. A retrospective study on 104 patients receiving trastuzumab shows that p53 status is not a predictor of the clinical efficacy [90].

Some studies suggested that p53 may influence response to antihormonal treatments. *TP53* mutations are less frequent in patients with ER-positive breast cancers, but they are associated with a poorer prognosis in these patients. *In vitro* studies on human breast cancer cell lines, MN1 (p53WT) and MDD2 (p53MUT) derived from MCF-7, it was shown that p53 mutated cells were more resistant to cytotoxic effects of 4-hydroxy-tamoxifen compared to p53 wild-type cells [91]. Clinical studies on patients with locally advanced breast cancer treated with tamoxifen or primary chemotherapy showed that mutations in the *TP53* gene are associated with a poor survival [92]. In a meta-analysis of 4,683 patients with breast cancer, the overexpression of p53 was correlated with poor outcome in premenopausal women treated with tamoxifen after chemotherapy [93].

7.3. p53 and Radiotherapy. Tumor cell death following exposure to radiotherapy occurs by apoptosis and is a p53-dependent event [94]. Preclinical studies were realized on immunocompromised mice engrafted with fibrosarcoma tumors expressing a functional or *TP53*-deficient gene. Tumors with functional *TP53* contained a large proportion of apoptotic cells and regressed after treatment with gamma radiation or adriamycin. p53-deficient tumors treated with the same regimens continued to enlarge and contained few apoptotic cells. Reduced levels of functional p53 would prevent radiotherapy-induced cell death, while mutant p53 is a marker for resistance. The defects in apoptosis due to inactivation of p53 can produce treatment-resistant tumors, suggesting that p53 status could be important in determining tumor response [95].

In conclusion, *TP53* status shows a strong prognosis impact and this could be useful in the choosing the best treatment for breast cancer. Generally, *TP53* mutated is associated with a poor response to chemotherapy, hormonotherapy or radiotherapy. Discordant studies concerning its predictive value exist, and this is linked to method of detection of *TP53* status. We show that FASAY test and sequencing of *TP53* are better than immunohistochemistry to determine if *TP53* is mutated or not. Prospective studies using these two methods could better determine its predictive value according to response to treatments.

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